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Induction of δ-Aminolevulinic Acid Formation in Etiolated Maize Leaves Controlled by Two Light Systems[1]

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ABSTRACT

A short illumination of etiolated maize (*Zea mays*) leaves with red light causes a protochlorophyll(ide)-chlorophyll(ide) conversion and induces the synthesis of δ-aminolevulinic acid (ALA) during a subsequent dark period. In leaves treated with levulinic acid, more ALA is formed in the dark than in control leaves. Far red light does not cause a conversion of protochlorophyll(ide) into chlorophyll(ide) and does not induce accumulation of ALA in the dark. Both red and far red preilluminations cause a significant potentiation of ALA synthesis during a period of white light subsequent to the dark period. The results indicate a dual light control of ALA formation. The possible role of phytochrome and protochlorophyllide as photoreceptors in this control system is discussed.

Chlorophyll synthesis in higher plants involves the light-induced formation of ALA[2] (4) by an enzyme system which, in all probability, is not identical with ALA synthetase (1, 10). In previous papers we presented evidence that light induces the synthesis, rather than the activation of this ALA-producing enzyme(s) with a half-life of about 80 min (3, 8). It was also shown that the cessation of ALA accumulation after a light-dark transition in leaves treated with LA, a specific inhibitor of the ALA dehydratase, is in agreement with the proposed half-life of the enzyme (3). The rapid cessation of PChl synthesis in the dark after a light period in leaves not treated with LA could not be explained by the properties of the enzyme system alone and suggested a feedback inhibition of the activity of the ALA-producing enzyme, which could be removed by an additional illumination. It was thus suggested that light may affect ALA synthesis in two ways: (*a*) by inducing the synthesis of the enzyme(s) required for its formation; and (*b*) by removal of an inhibitor affecting the activity of the enzyme system. In this paper we shall present further evidence that ALA synthesis in etiolated maize leaves is regulated by two light systems. The evidence indicates, in agreement with the suggestion by Masoner and Kasemir (9), that the pigments involved may be phytochrome and PChl.

MATERIALS AND METHODS

The primary leaves of 10- to 11-day-old etiolated *Zea mays* seedlings, grown at 25 C from seed on vermiculite in a dark room, were used throughout the experiments. The leaves were cut 6 cm below their tops under a weak green safelight, weighed in batches of 1 to 2 g, and then placed in a single row with their bases in transport cuvettes, 0.6 cm wide and 2.5 cm high, containing either distilled H_2O or a 50 mM LA solution, brought to pH 5.8 to 6.0 with KOH. After a 2-hr preincubation period in the dark, the leaves were exposed for 10 min to light from a Xenon source, filtered through water-cooled interference filters (Intraflex B40, Balzers, Liechtenstein). Light intensity was measured with an YSI Kettering model 65 radiometer, and is expressed as erg·cm^{-2}·sec^{-1}. After the illumination, the leaves were returned to the dark for 4 hr and, when required, exposed again to white fluorescent light of 1.2×10^3 erg·cm^{-2}·sec^{-1} (80 ft-c) for 3 hr. Concomitantly, etiolated leaves treated with or without LA were kept in the dark for 0 hr, 2 hr, 4 hr, and 7 hr, or exposed for 3 hr to white light without preillumination. Some of the samples were then extracted with 80% acetone and PChl and Chl determined spectrophotometrically, while from others trichloroacetic acid extracts were prepared for ALA determinations, as described earlier (8, 10). From the PChl content of the etiolated leaves ($PChl_0$) and that of Chl (Chl_P) at the end of the 4-hr dark period following preillumination the amount of PChl resynthesized in the dark ($PChl_S$) was calculated: $PChl_S = Chl_P + PChl_P - PChl_0$. It had previously been established that the level of Chl_P remained constant during the 4 hr of darkness. PChl and Chl contents are expressed as nmol ALA/g fresh weight of the leaves (8 mol ALA = 1 mol Chl or PChl). Per cent PChl-Chl transformation is defined as $Chl_P \times 100/PChl_0$. Chl and ALA content were also determined at the end of the 3-hr period of continuous illumination.

The pyrrolle formed with acetylacetone was identified as ALA-pyrrolle on thin layer chromatograms (5).

RESULTS

Effect of Short Illumination on PChl-Chl Conversion in Etiolated Leaves and on ALA Accumulation during a Subsequent Dark Period. Detached maize leaves treated with LA were exposed for 10 min to various intensities of light filtered through interference filters and returned for 4 hr to the dark. Figure 1 shows the dose response curves for the PChl-Chl conversion which occurred during illumination with light filtered through filters with maximal transmissions at 650 nm (R_{650}) and 659 nm (R_{659}). Figure 2 gives the dose response curve for total ALA accumulation (PChl + free ALA) during the subsequent 4-hr dark period, which was induced by R_{650} and R_{659}. From these and similar dose response curves, the relative efficiency of some wavelengths in the 620 nm–725 nm region ($R_{620-725}$) in respect to 55% PChl-Chl transformation and accumulation of 70 nmol ALA was calculated (Fig. 3). Among the wavelengths investigated, R_{650} and R_{659} were most and equally effective in causing PChl-Chl conversion, while in the same experiments about 50% more ALA accumulated after illumination with R_{659} than with R_{650}. This indicates that the action spectra for PChl-Chl conversion and for induction of ALA synthesis are not identical. In R_{700}

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[2] Abbreviations: PChl: protochlorphyll(ide); Chl: chlorophyll(ide); ALA: δ-aminolevulinic acid; LA: levulinic acid; FR: far red light; R: red light.

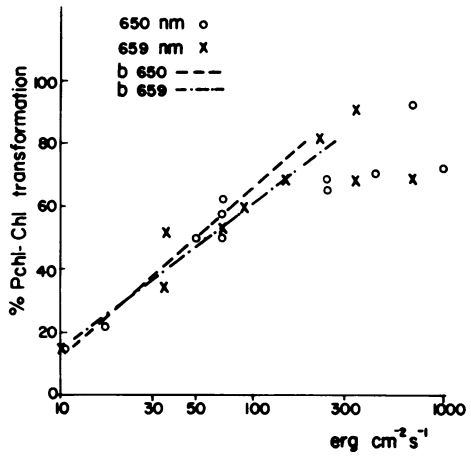

FIG. 1. Dose response curves and their regression lines (b) for the per cent transformation of PChl into Chl during a 10-min illumination with light of 650 nm and 659 nm in detached etiolated maize leaves treated with 50 nmol LA.

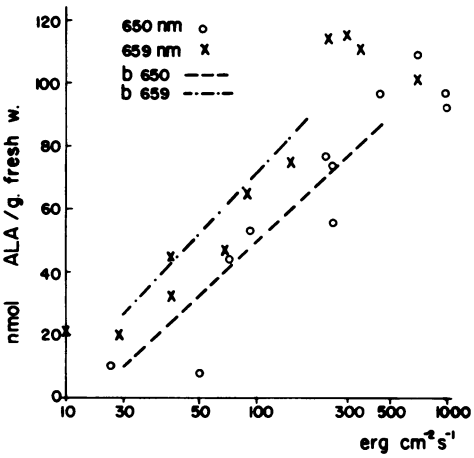

FIG. 2. Dose response curves and their regression lines (b) for induction by 10-min light of 650 nm and 659 nm of total ALA during 4 hr of darkness in detached etiolated maize leaves treated with 50 nmol LA.

or R_{725} no PChl-Chl conversion occurred in light intensities below 1,000 erg·cm^{-2}·sec^{-1}; at higher intensities 0 to 10% conversion occurred, but was not followed by ALA accumulation in the dark. Control leaves kept for 4 hr in the dark did not accumulate ALA. Maximal PChl transformation obtained with R_{650} or R_{659} varied between 70 to 80% and up to 100 to 120 nmol ALA/g fresh weight accumulated in the dark; its induction by R_{659} was saturated at about 300 erg·cm^{-2}·sec^{-1}.

The etiolated maize leaves used in the experiments contained PChl equivalent to 72 ± 3 nmol ALA/g fresh weight. In control leaves (not treated with LA) illuminated with 250 erg·cm^{-2}·sec^{-1} at R_{650} PChl-Chl conversion was not significantly different from that in LA-treated leaves. PChl resynthesized during the following dark period amounted to about 33 ± 4 nmol ALA (Table I). In addition to the converted Chl, at the end of the experiment the leaves again contained about 80% of their initial PChl content. In the LA-treated leaves (due to the inhibitive effect of LA on the ALA dehydratase), less PChl accumulated than in the controls and at the end of the dark period the leaves contained only about 50% of their initial PChl content. However, due to the "free" ALA which accumulated in the LA-treated leaves in the dark after light treatment, their "total" ALA content was always higher than in the controls (Table I). This difference in ALA accumulation in the dark between leaves with or without LA treatment recalls the earlier report that ALA accumulation is higher in LA-treated leaves returned to the dark after 4 hr of light, than in nontreated controls (3).

Effect of Preillumination with R_{650} and FR_{725} on ALA Production in White Light, following a Dark Period. Etiolated control leaves accumulated Chl equivalent to 176 nmol ALA/g fresh weight (Table I) during 3 hr under white fluorescent light of 80 ft-c. Since the same amount of "total" ALA (Chl + ALA) was produced in LA-treated leaves under the same light conditions, the data obtained with this system are relevant to the problem of Chl synthesis and control (8).

A 10-min preillumination with R_{650} 4 hr before a 3-hr treatment with white light increased total ALA production significantly during the light period in leaves incubated with or without ALA. Figure 4 shows the effect of varying light intensity of R_{650} on accumulation of total ALA in LA-treated leaves during the 3-hr light period. There is an increase in potentiation with increase in light intensity up to 50 erg to 100 erg·cm^{-2}·sec^{-1}, indicating that potentiation is light-saturated at relatively low light intensities.

Potentiation was also obtained with R_{725} (Fig. 5). The FR light had no effect below intensities of 300 erg·cm^{-2}·sec^{-1}, and maximal potentiation at this wavelength was obtained with 1,000 erg·cm^{-2}·sec^{-1}. At higher intensities potentiation decreased somewhat. Thus, preillumination with R_{650}, but not R_{725} affects ALA synthesis in the dark, while both R_{650} and R_{725} potentiate ALA synthesis subsequently in light (Fig. 6). A similar potentiation of ALA synthesis in light by R and FR pretreatments has also been reported by Masoner and Kasemir (9).

Attempts to reverse the R_{650} effect by a subsequent exposure of the leaves to R_{725}, using various combinations of light energies, have so far been unsuccessful. In all cases ALA production after R_{650} and R_{725} was equal to that after R_{650} treatment alone.

DISCUSSION

A short illumination with R causes a PChl-Chl converion and induces the synthesis of ALA during a subsequent 4-hr dark period. Far red does not induce PChl-Chl conversion and does not induce accumulation of ALA in the dark. Both light treatments cause a significant potentiation of ALA synthesis during a subsequent period of continuous white light. Evidently, both light treatments induce changes in the dark which lead to an increase in ALA formation during the following 3-hr period of continuous light. The increase of ALA production in the light is not due to formation of committed precursors (ALA or porphyrins), since no ALA accumulates in the dark in LA-treated leaves after a FR illumination. Neither can the potentiation of ALA production in the light be dependent solely on the PChl-

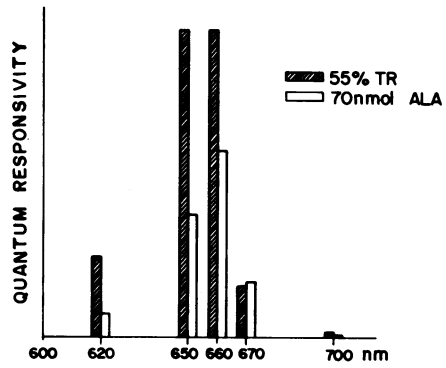

FIG. 3. Relative quantum efficiency of various wavelengths for 55% PChl-Chl transformation (TR) and for the production of 70 nmol ALA (PChl + free ALA) during 4 hr of darkness in etiolated maize leaves treated with 50 nmol LA.

Table I. The effect of Red and Far-Red illumination on the synthesis of ALA in etiolated maize leaves

Detached etiolated maize leaves were placed with their bases in water (W) or in a solution containing 50nmol LA. The leaves were illuminated for 10 min with R (650nm) or FR (725nm) light, placed for 4 hr in the dark and were then exposed for 3 hr to white light (80 ft-c). Protochlorophyll content of the etiolated leaves prior to preillumination: 72 ± 3 nmol ALA/g fresh wt. The amounts of synthesized PChl, Chl and free ALA are expressed in ALA nano equivalents/g fresh wt and were calculated as described in "Methods."

Treatment		10 min pre-illumination		% PChl-Chl conversion	Synthesis during 4 hr Dark			Synthesis during 4 hr Dark + 3 hr Light			Synthesis of total ALA[3] due to preillumination	
LA	W	λ nm	erg·cm⁻²·s⁻¹		PChl[1]	free ALA[1]	total ALA[1]	Chl[1]	free ALA[1]	total ALA[3]	3 hr Light	4 hr Dark + 3 hr Light
	+	..	..					176 ± 18	0	176 ± 18		
..		..	..					61 ± 8	118 ± 9	180 ± 6		
	+	650	250	66 ± 3[2]	33 ± 4	0	33 ± 4	376 ± 36	0	376 ± 36	167	200
+		650	250	52 ± 2	9 ± 3	74 ± 8	83 ± 8	101 ± 13	302 ± 5	403 ± 22	163	225
	+	725	1000	6 ± 1	0	0	0	268	0	268	92	92
+		725	1000	3	0	0	0	62 ± 16	232 ± 2	294 ± 30	114	114

[1] nmol ALA/g fresh wt

[2] Standard error

[3] Chl + PChl + free ALA

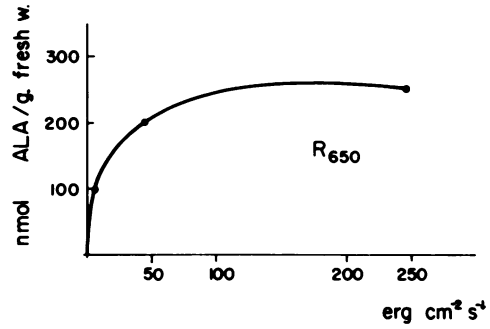

FIG. 4. Increase in accumulation of total ALA (Chl and free ALA) during 3 hr of white light (80 ft-c) as a function of preillumination with R (650 nm) light of various intensities. Etiolated maize leaves treated with 50 nmol LA were preilluminated 4 hr before their exposure to white light.

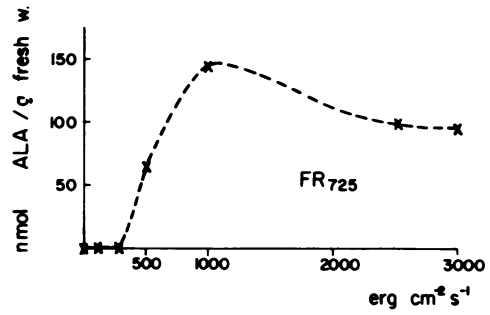

FIG. 5. Increase in accumulation of total ALA during 3 hr of white light (80 ft-c) as a function of preillumination with FR (725 nm) light. Etiolated maize leaves treated with 50 nmol LA were preilluminated 4 hr before their exposure to the white light.

Chl conversion during the preillumination, since no such conversion occurs in FR. The results indicate a dual effect of light on ALA formation. In earlier papers, evidence was presented that the ALA-producing enzyme(s) is labile and that light is apparently required for its synthesis (3, 8) and/or that of a later precursor for PChl. It is suggested that R and FR induce the synthesis of the ALA-producing enzyme(s), but that activity of the enzyme is inhibited through feedback by a critical level of PChl or an earlier precursor, the concentration of which is regulated by the level of PChl. The enzyme induced by R would produce ALA in the dark, because of the PChl-Chl transformation caused by the R. FR would induce enzyme synthesis, but the enzyme would not be active in the dark, since the inhibitive level of the PChl is not changed by FR. When subsequently exposed to white light, higher initial rates of ALA synthesis are expected in the preilluminated leaves due to the enzyme synthesis, which occurred during the preceding dark period as a consequence of the preillumination.

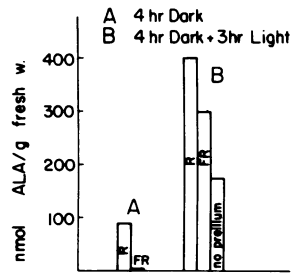

FIG. 6. Accumulation of total ALA (PChl + free ALA) during 4 hr of darkness (A) and a following 3-hr period in continuous white light (B) due to preillumination. Etiolated maize leaves were treated with 50 nmol LA and preilluminated for 10 min with 250 erg·cm^{-2}·sec^{-1} of R (650 nm; R) or with 10^3 erg·cm^{-2}·sec^{-1} of FR (725 nm; FR).

The light acceptor for the system controlling "activity" of the ALA-producing enzyme thus appears to be some form of PChl, the pigment responsible for the PChl-Chl conversion. The light acceptor for the synthesis of ALA-forming enzyme (which in all probability is not identical with the ALA synthetase [1, 10]), is activated by both R and FR.

A rapid accumulation of Chl in maize leaves in light after preillumination with R or FR was reported earlier by Raven (11) who found it difficult to reverse the effect of R by a following FR treatment. The same holds for pea seedlings, where a partial reversal by FR could be obtained only if the induction was performed with an extremely low dosage of R. In cotyledons of the mustard seedling both R and FR accelerate regeneration of PChl in the dark after a flash of white light, but the effect of R is partially reversed by a following FR treatment (6). A similar reversible R-FR effect on increase of ALA accumulation in mustard cotyledons in continuous white light has been reported by Masoner and Kasemir (9). The effect of the preillumination on regeneration of PChl, or accumulation of Chl and/or ALA has been attributed to the direct (6, 9) or indirect (8) effect of phytochrome. In contrast to the situation in the mustard seedling, there is no evidence that the ALA-forming system in the 11-day-old etiolated maize leaves is already well developed, but inhibited; rather there is evidence for its light-induced synthesis (3).

A dual effect of light, mediated through phytochrome and PChl, on induction of ALA synthesis has been proposed by Masoner and Kasemir (9) for the cotyledons of mustard seedlings and by Fluhr *et al.* (3) for maize leaves. Evidence for dual light control has also been presented for lag elimination for Chl synthesis in *Euglena* (12). It has been suggested that in this alga, which does not show phytochrome activity, light control is exercised via PChl and another nonplastid photoreceptor.

The effect of light on Chl synthesis can be properly approached only in the larger framework of light-induced plastomorphogenesis. The evidence for possible dependence of Chl synthesis on the light-induced synthesis of thylakoid membrane

proteins has been summarized by Kirk (7). Although we regard the ALA-making enzyme as nonstable with a half-life of about 80 min (3), the proposed model of dual light control of ALA synthesis does not exclude regulatory interrelationships between Chl and other plastid constituents. A possible effect on enzyme induction by phytochrome is also compatible with the recently described action of this pigment on plastid membrane permeability (2).

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